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Allelotype of Uterine Leiomyomas

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ABSTRACT: *Uterine leiomyomas are the most common benign tumor that arise from smooth muscle cells of the myometrium. Little is known about the etiology and pathogenesis of this tumor. To investigate the molecular pathogenesis of these tumors, we have conducted an allelotype of 102 leiomyomas from 12 patients, using 67 fluorescently-tagged oligonucleotide primers amplifying microsatellite loci covering all autosomes. No areas of the genome showed frequent loss of heterozygosity (LOH); however, the highest rate of LOH (9%) was observed on 7q, consistent with previous cytogenetic observations. Uterine leiomyomas are sometimes multiple. In general, multiplicity of other types of neoplasm is associated with genetic predisposition to the disease. Because multiple tumors were available from each of the 12 patients studied, we looked for evidence of allele-specific LOH, which might indicate the presence of an underlying predisposition gene. However, no evidence for allele-specific LOH was detected, indicating that if cases of multiple uterine leiomyoma are due to an underlying predisposition gene, it is unlikely to be a recessive oncogene.*

INTRODUCTION

Uterine leiomyomas (fibroids) are present in 20–30% of women over age of 30 years [1]. They are frequently multiple and may attain a large size, causing severe pain, excessive bleeding, and infertility. They are the leading cause of hysterectomy [2]. Uterine leiomyomas appear to be hormonally regulated, as they only arise after puberty and tend to atrophy after menopause [3]. However, despite the importance of this tumor to women's health, little is known about its etiology, pathogenesis, or genetic basis of development.

Previous cytogenetic studies of uterine leiomyoma have revealed that about 40% have chromosomal abnormalities [4–11]. The most common chromosome aberrations are interstitial deletions of 7q [12–15]. Molecular genetic analyses have confirmed the presence of loss of heterozygosity (LOH) on 7q21∼32 in uterine leiomyomas [16–18]. Rearrangements of 12q13∼q15, mainly as t(12;14), characterize the second cytogenetically abnormal subgroup, followed by nonrandom involvement of 6p21∼23, 13q13∼22, 3q and chromosome 1 [9, 19]. However, there have been few studies describing LOH in uterine leiomyomas elsewhere in the genome.

There is some evidence that there is an excess familial risk of developing uterine leiomyomas in first-degree relatives of affected individuals, and therefore, that a heritable predisposition to this disease exists [20]. Currently, however, the nature of this familial predisposition is ill defined. Further study of the susceptibility syndrome, and in particular the localization and identification of the genes that may be responsible, is likely to be complicated by the high frequency of sporadic uterine leiomyomas in the general population, and by the fact that many women with the disease are asymptomatic. Both of these factors will limit the power of pedigrees analyzed for genetic linkage to locate the underlying genes.

As is the case in many syndromes of familial predisposition to neoplasia, some patients with uterine leiomyoma develop multiple independent tumors. For the purposes of susceptibility gene localization, this feature offers an alternative approach to conventional genetic linkage analysis of families with multiple affected cases. If the underlying susceptibility gene is a tumor suppressor gene, there will usually be LOH in a substantial proportion of tumors in the vicinity of the susceptibility gene, as previously observed for *BRCA1* and *BRCA2* [21, 22]. Where there are multiple tumors in the same individual, each tumor that loses heterozygosity should lose the same allele, which is inherited from the nonmutation-carrying parent. This pattern is well illustrated in the rare autosomal dominant predisposition to skin adnexal tumors, familial cylindromatosis, which has been localized to 16q. In this disease,

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Table 1 A summary of allelotyping of uterine leiomyomas

Loci	Genetic (cM)	Cytogenetic	No. informative (%)	No. showing LOH (%)
D1S214	16.4	1p36.31∼36.21	58 (57)	0 (0)
D1S197	78.3	1p33	82 (80)	0 (0)
D1S2691	197	1q23.3∼24.2	83 (81)	0 (0)
D1S2800	256.1	1q41∼42.13	65 (64)	1 (2)
D2S162	21.3	2p25.1	88 (86)	0 (0)
D2S391	73.8	2p16.3∼14	51 (50)	0 (0)
D2S112	145.8	2q21.1	93 (91)	0 (0)
D2S206	248.3	2q37.2∼37.3	88 (86)	0 (0)
D3S1597	24.1	3p24.3∼24.1	93 (91)	0 (0)
D3S1309	157.4	3q22-23	92 (90)	0 (0)
D3S1262	207.2	3q26.3∼27	93 (91)	0 (0)
D4S1599	22	4p16.1∼15.32	44 (43)	0 (0)
D4S1551	38.3	4p15.32∼15.1	102 (100)	0 (0)
D4S1586	146.4	4q28.3∼31.21	89 (87)	0 (0)
D5S432	21.4	5p15.31∼14.2	95 (93)	0 (0)
D5S1962	82.8	5q13.2∼14.1	89 (87)	0 (0)
D5S394	179.8	5q35.1∼35.2	34 (33)	0 (0)
D6S260	29.6	6p23∼22.3	85 (83)	0 (0)
D6S273	44.9	6p22.1∼21.31	85 (83)	0 (0)
D6S468	108	6q21	79 (77)	0 (0)
D6S264	179.1	6q27	69 (68)	0 (0)
D7S517	7.8	7p22.3	64 (63)	0 (0)
D7S516	42.1	7p15.2∼14.3	74 (73)	0 (0)
D7S492	100.5	7q21.11	81 (79)	3 (4)
D7S489	101	7q21.11	79 (77)	2 (3)
D7S669	105	7q21.13	85 (83)	3 (4)
D7S440	108–112	7q21.12∼21.3	61 (60)	2 (3)
D7S518	112.9	7q21.13∼21.3	102 (100)	9 (9)
D7S657	120	7q21.3∼22.1	96 (94)	4 (4)
D7S496	120.7	7q21.3∼22.1	83 (81)	4 (5)
D7S466	131.4	7q22.1∼22.2	82 (80)	4 (4)
D7S471	142–143	7q31.31∼31.32	91 (89)	4 (4)
D7S530	157	7q31.33∼34	54 (53)	0 (0)
D8S298	42.7	8p21.2∼12	63 (62)	0 (0)
D8S281	122.6	8q23.3∼24.12	45 (44)	0 (0)
D9S259	45.1	9p13.3∼13.2	90 (88)	0 (0)
D9S290	141.1	9q34.12∼34.3	102 (100)	0 (0)
D10S249	0	10p15.3	79 (77)	0 (0)
D10S539	75.4	10q21.1	66 (65)	0 (0)
D10S574	124.4	10q24.2∼25.1	38 (37)	0 (0)
D11S902	24.7	11p15.3∼15.2	91 (89)	0 (0)
D11S1328	128.4	11q23.2∼23.3	69 (68)	0 (0)
D12S94	1.1	12p13	75 (74)	0 (0)
D12S1635	66.8	12q11∼13	80 (78)	0 (0)
D12S105	118.9	12q22	77 (75)	0 (0)
D13S291	43.7	13q14.12∼14.13	48 (47)	0 (0)
D13S158	86.9	13q22.3∼32.1	66 (65)	0 (0)
D14S274	53.8	14q22.1∼23.3	60 (59)	0 (0)
D14S277	68	14q23.3∼24.1	69 (68)	0 (0)
D15S144	25.3	15q13.3	76 (75)	0 (0)
D15S158	84.8	15q25.2∼26.1	85 (83)	0 (0)
D16S423	8.4	16p13.33	61 (60)	0 (0)
D16S519	19.7	16p13.3∼13.13	69 (68)	0 (0)
D16S420	43.2	16p12.3∼12.1	87 (85)	0 (0)
D16S419	65.8	16q12.2∼22.1	68 (67)	1 (2)
D16S496	84.4	16q22.1∼23.1	30 (29)	0 (0)
D16S516	98.3	16q22.1∼23.1	71 (70)	0 (0)
D17S786	18.1	17p13.2∼13.1	87 (85)	0 (0)
D17S802	108.2	17q24.3∼25.3	75 (74)	0 (0)
D18S53	40.4	18p11.23∼11.22	92 (90)	0 (0)
D18S64	83	18q21.2∼21.32	70 (69)	0 (0)

(*Continued*)

Table 1 Continued

Loci	Genetic (cM)	Cytogenetic	No. informative (%)	No. showing LOH (%)
D19S424	10.8	19p13.3	92 (90)	0 (0)
D19S418	97.5	19q13.43	68 (67)	0 (0)
D20S95	16.4	20p12.3∼12.2	81 (79)	0 (0)
D20S109	73.6	20q12∼13.12	85 (83)	0 (0)
D21S1260	51.6	21q22.3-qter	44 (43)	0 (0)
D22S315	16.2	22q11.2	97 (95)	0 (0)

multiple tumors develop, approximately two thirds show loss of heterozygosity in the vicinity of the susceptibility locus, and all cylindromas showing allele loss lose the haplotype of 16q markers inherited from the nonmutation-carrying parent [23, 24]. We have termed this phenomenon, allele-specific loss of heterozygosity (ASLOH). Elsewhere in the genome there may also be LOH, and it may even occur at high frequency, but it will not usually be allele specific. Use of ASLOH in individuals with multiple tumors offers the advantage of obviating the requirement for ascertainment of multiple cases from the same family, and hence is most applicable to those susceptibility syndromes in which multiplicity of the neoplasm is common but in which the manifestations are variable. Moreover, because LOH in tumors often spans large chromosomal distances, the marker map used in an ASLOH search for a susceptibility gene can be less dense than the 10–20 cM map that is usually employed in conventional genetic linkage analyses. Although, to our knowledge, ASLOH has never been used as the sole method of susceptibility gene localization, it was employed as one of several approaches in the localization of the gene for Peutz-Jegher's syndrome, *LKB1*, to 19p [25].

To investigate further the molecular defects in uterine leiomyomas, and to evaluate the possibility of detecting a susceptibility gene by ASLOH, we have conducted an allelotype of 102 leiomyomas from 12 patients.

MATERIALS AND METHODS

Uterine Leiomyoma Specimens and DNA Extraction

High molecular weight DNA was isolated from 94 fresh, frozen leiomyomas and matched normal tissues (cervix) from 11 patients with uterine leiomyomas using standard methods. Formalin-fixed, paraffin-embedded sections from an additional 8 tumors from a single patient were incubated in 10 mM Tris HCl (pH 7.5), 1 mM EDTA, 1% (w/v) SDS, and 500 μg/mL proteinase K at 37°C for 72 hours. The mixture was then heated at 100°C for 10–15 minutes to inactivate the proteinase K, and was used directly in polymerase chain reaction (PCR).

Primers and PCR

The genetic and cytogenetic maps of the 67 microsatellite loci used in this study are shown in Table 1 [26–31]. At least two markers per autosome were used with greater numbers for the larger chromosomes. Polymerase chain reaction amplification of microsatellite loci was conducted using fluorescently-tagged oligonucleotide primers (GENSET) in an Omnigene thermal cycler (Hybaid) (Table 1). The reaction mixture (total 15 μl) consisted of 1.5 μl of 10 × PCR buffer, 25 mM $MgCl_2$, 1.5 μl (2 mM each nucleotide) of dNTPs, 0.3 μl (5 OD U/mL) of each primer, 0.15 μl (10 mg/mL) of BSA, 0.1 μl of Taq polymerase, 9.25 μl of water, and 1 μl of DNA. Polymerase chain reactions conditions were: 35–40 cycles of denaturation at 94°C for 1 minute, annealing at the appropriate temperature (50–60°C) for 1 minute, and extension at 72°C for 1 minute, followed by a final extension for 5 minutes at 72°C. Analyses suggestive of LOH were repeated at least once to confirm.

Electrophoresis and Data Analysis

The PCR products were analyzed on 4.5% polyacrylamide denaturing gels (Anachem, Leciester) in 1 × TBE (89 mM Tris-borate, 2 mM EDTA pH 8.0) buffer using an ABI 377 automated fluorescent DNA sequencer (Applied Biosystems, Foster City, CA, USA), which has a four-color detection system. Two microliters of each PCR reaction was combined with 2 μl formamide and 0.5 μl of a TAMRA fluorescent size marker (GS500, Applied Biosystems). This mix was denatured for 6 minutes at 94°C, after which 1.5 μl was loaded into each well on a prewarmed gel. Gels were run for 2.5 hours at 200 watts power, 60 mamps current, 2,900 volts voltage, 51°C temperature, and scan rate of 2,400 scans/hour. While the samples were undergoing electrophoresis, the fluorescence was detected in the laser scanning region using filter set C, and was collected and stored using the GeneScan Collection Software 1.1 (Applied Biosystems). The fluorescent gel data collected during the run was analyzed using GeneScan Analysis Software 2.0.2 (Applied Biosystems) at the end of the run. Each fluorescent peak was quantitated in terms of allele, peak height, and peak area. The results were then imported into Genotyper (version 1.1) (Applied Biosystems) for further analysis and printing.

Assessment of LOH by Calculating Allele Ratios

The comparison of the ratios between tumors and their controls was done using the formula T1:T2/N1:N2, where T1 and N1 are the area under peak (AUP) of the smaller allele for the tumor and normal samples, respectively, and T2 and N2 are the AUP of the larger alleles. For ratios greater than 1, the reciprocal of ratio is calculated to give a value between 0.00 and 1.00. A value of 0.25 or less was assigned as indicative of LOH.

Table 2 LOH on 7q21∼31 in uterine leiomyomas[a]

Case no.	D7S517	D7S516	D7S492	D7S489	D7S669	D7S440	D7S518	D7S657	D7S496	D7S466	D7S471	D7S530
F2A	—	—	—	N	—	N	—	—	—	—	—	N
F2B	—	—	—	N	—	N	—	—	—	—	—	N
F2C	—	—	—	N	—	N	—	—	—	—	—	N
F2D	—	—	—	N	—	N	—	—	—	—	—	N
F2E	—	—	—	N	—	N	—	—	—	—	—	N
F2F	—	—	—	N	—	N	—	—	—	—	—	N
F2G	—	—	—	N	—	N	—	—	—	—	—	N
F2H	—	—	2	N	1	N	1	2	1	1	2	N
F2I	—	—	—	N	—	N	—	—	—	—	—	N
F2J	—	—	—	N	—	N	—	—	—	—	—	N
F2Cer[b]	—	—	—	N	—	N	—	—	—	—	—	N
F5A	—	—	—	—	—	—	—	—	N	—	—	N
F5B	—	—	1	1	2	1	2	1	N	2	1	N
F5C	—	—	—	—	—	—	—	—	N	—	—	N
F5D	—	—	2	2	1	2	1	2	N	1	2	N
F5E	—	—	—	—	—	—	—	—	N	—	—	N
F5F	—	—	—	—	—	—	—	—	N	—	—	N
F5G	—	—	—	—	—	—	—	—	N	—	—	N
F5H	—	—	—	—	—	—	1	—	N	1	2	N
F5I	—	—	—	—	—	—	—	—	N	—	—	N
F5J[b]	—	—	—	—	—	—	—	—	N	—	—	N
F6A[b]	N	—	—	N	—	—	—	N	N	—	—	N
F6B	N	—	—	N	—	—	—	N	N	—	—	N
F6C	N	—	—	N	—	—	—	N	N	—	—	N
F6D	N	—	—	N	—	—	—	N	N	—	—	N
F6E	N	—	—	N	—	—	2	N	N	—	—	N
F6F	N	—	—	N	—	—	—	N	N	—	—	N
F6G	N	—	—	N	—	—	—	N	N	—	—	N
F10A[b]	—	N	—	N	—	—	—	N	—	—	—	—
F10B	—	N	—	N	—	—	—	N	—	—	—	—
F10C	—	N	—	N	—	—	—	N	—	—	—	—
F10D	—	N	—	N	—	—	—	N	—	—	—	—
F10E	—	N	—	N	—	—	—	N	—	—	—	—
F10F	—	N	—	N	—	—	—	N	—	—	—	—
F10G	—	N	—	N	—	—	—	N	—	—	—	—
F10H	—	N	—	N	—	—	2	N	1	—	—	—
F10I	—	N	—	N	—	—	2	N	1	—	—	—
F10J	—	N	—	N	—	—	2	N	—	—	—	—
F11A	—	N	—	—	—	—	—	—	—	—	—	N
F11B	—	N	—	—	—	—	—	—	—	—	—	N
F11C	—	N	—	—	—	—	—	—	—	—	—	N
F11D	—	N	—	—	—	—	—	—	—	—	—	N
F11E	—	N	—	—	—	—	—	—	—	—	—	N
F11F	—	N	—	—	—	—	—	—	—	—	—	N
F11G	—	N	—	—	—	—	—	—	—	—	—	N
F11H	—	N	—	—	—	2	2	1	2	—	—	N
F11I	—	N	—	—	—	—	—	—	—	—	—	N
F11J	—	N	—	—	—	—	—	—	—	—	—	N
F11K	—	N	—	—	—	—	—	—	—	—	—	N
F11L	—	N	—	—	—	—	—	—	—	—	—	N
F11N[b]	—	N	—	—	—	—	—	—	—	—	—	N

Abbreviations: N, non-informative, —, informative but no LOH.

[a]LOH of allele 1 to n.

[b]Matched normal control issue.

RESULTS

The numbers of uterine leiomyomas showing LOH are shown in Table 1. Loss of heterozygosity was not observed at high frequency in any part of the genome. It was most frequently seen on 7q21∼31 and very occasionally on other autosomes. By typing additional markers, it appears that the LOH on 7q centers around *D7S518* and often does not involve the whole chromosome or the whole of the long arm (Table 2). No leiomyoma showed LOH on more than one chromosome.

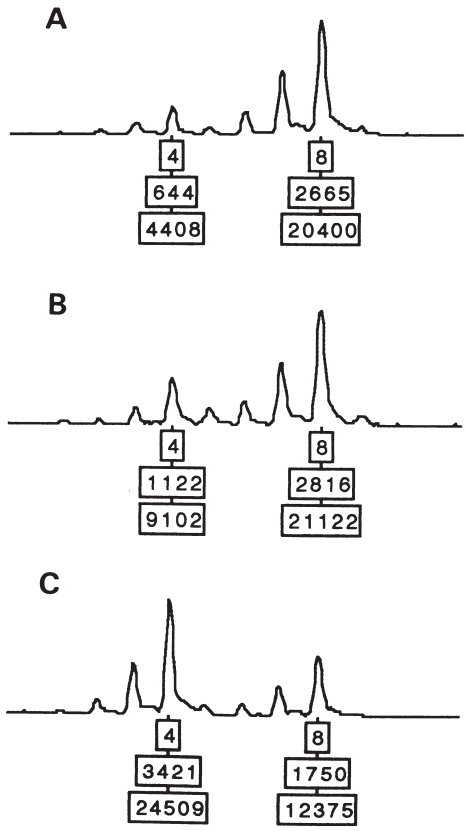

Figure 1 Fluorescent peaks of the gel of fibroids (A) F10H, (B) F10I, and (C) F10J at D7S496. Each peak was quantitated in terms of allele 1 and 2 (no. 4 and 8), peak height (644, 2665, 1122, 2816, 3421, and 1750), and peak area (4408, 20400, 9102, 21122, 24509, and 12375). Both F10H and F10I show LOH of allele 1 (no. 4) of D7S496.

This LOH analysis included several uterine leiomyomas from each individual studied; therefore, we were interested in evaluating the utility of ASLOH to localize a susceptibility gene for uterine leiomyomata. Loss of heterozygosity at a particular locus in more than one uterine leiomyoma from the same individual was only observed on 7q. F10H, F10I, and F10J showed loss of the same allele at *D7S518*, which extended to F10H and F10I at *D7S466.* By contrast, F5B clearly demonstrates loss of a different marker haplotype on 7q to F5D and F5H (Table 2) (Fig. 1).

DISCUSSION

Previous cytogenetic studies have shown that up to 40% of leiomyomas contain chromosome aberrations [4–11], with the most common reported site of deletion being on 7q. The majority of leiomyomas with a cytogenetically visible 7q deletion show LOH on 7q, while only a small proportion with a normal 7q karyotype exhibit LOH in this region [13]. In the single published study by comparative genomic hybridization, only 2 of 14 of uterine leiomyomas exhibited DNA sequence copy number changes, including losses on chromosomes 4 and 1 (no losses on chromosome 7) and gains on chromosomes 14 and 19 [18].

We have analyzed LOH through all of the autosomes in 102 leiomyomas from 12 patients, and this is the first full allelotype of uterine leiomyomas. Polymerase chain reaction was repeated twice to three times for those cases showing LOH. Consistent with previous cytogenetic reports, we observed 7q as the most common site of LOH, although the frequency of LOH in this region is still not high when compared to LOH frequencies elsewhere in the genome seen in other benign or malignant neoplasms. The LOH on 7q appears to be centered around *D7S518*, and commonly appears to be due to interstitial deletions rather than absence of the whole chromosome 7 or of the complete long arm. Loss of heterozygosity on 7q, suggesting the presence of a tumor suppressor gene(s), has been observed in various other neoplasms, including those of the prostate, breast, colon, and ovary [12–15, 32–36], but the identity of this putative gene(s) remains unknown. Loss of heterozygosity was observed only very occasionally elsewhere in the genome. However, it is conceivable that the density of the marker map used may miss some small areas of LOH similar to that observed on 7q. Overall, the allelotype adds further weight to the possibility of a tumor suppressor gene located on 7q that is mutated in a minority of leiomyomas, but does not provide strong evidence for similar genes located elsewhere in the genome.

Evidence exists for a familial risk of uterine leiomyomata, although further studies are warranted to confirm the preliminary observations. Such a susceptibility gene may, however, be difficult to study because of frequent phenocopies and common failure to develop symptoms despite the development of tumors. Moreover, analysis could be further complicated if the predisposition is due to multiple genes acting either additively or multiplicatively. Since multiplicity of tumors is commonly a feature of cancer predisposition syndromes, we made the hypothesis that individuals with multiple leiomyomas might carry a mutated allele of a uterine leiomyoma predisposition gene. The use of ASLOH in this context is predicted to be useful because individuals who have many tumors can, on their own, generate substantial evidence in favor of a susceptibility locus, and thus reduce the problems of genetic heterogeneity, multiplicatively-acting genes, sporadic cases, and lack of symptomatic penetrance. Moreover, the rate of LOH at a susceptibility locus is usually much higher in tumors that arise due to a predisposing gene mutation than in sporadic neoplasms of the same type. Therefore, even if multiple genes are acting in a single individual, and hence some tumors do not develop as a result of a particular susceptibility gene mutation, or if one or more of several leiomyomata arise due to chance rather than due to that particular susceptibility gene, evidence against that susceptibility locus will be diluted, because loss of the allele linked to the susceptibility gene mutation in the "sporadic" tumor(s) is relatively unlikely to occur.

In this study, however, there were low rates of LOH throughout the genome, indicating that the presence of an

underlying tumor suppressor type of susceptibility gene is unlikely. Moreover, even if a susceptibility gene associated with a relatively low rate of LOH was operative, the low levels of LOH render the ASLOH analysis relatively uninformative and reduce its power to establish the location of a gene. Finally, within the region on 7q where the highest rate of LOH was observed, there was little evidence in favor of ASLOH, as different tumors from individual F5 lost different 7q haplotypes.

The failure of the ASLOH approach to locate a susceptibility gene for uterine leiomyomata may indicate that our underlying assumption concerning the presence of a susceptibility gene in individuals with multiple uterine leiomyomas is erroneous, that the susceptibility gene is not of the tumor suppressor type, that our marker map is not dense enough to detect high levels of LOH restricted to relatively small genomic regions, or that the levels of LOH associated with the locus are too low and frequency of sporadic leiomyomata too high to detect the locus. Nevertheless, the use of the ASLOH strategy to locate a susceptibility gene may be more fruitful in the future in other conditions associated with multiple independent neoplasms.

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REFERENCES

1. Whitfield CR, eds (1986): Dewhurst's Textbook of Obstetrics and Gynecology for Post-Graduates, 4th ed. Blackwell Scientific Publications, London, pp. 726–733.
2. Clark-Pearson DL, Dawood MY (1990): Green's Gynecology: Essentials of Clinical Practice. Little Brown, and Co., Boston.
3. Baird DT, Bramley TA, Hawkins TA, Lumsden MA, West CP (1989): Effect of treatment with LHRH analogue Zoladex on binding of oestradiol, progesterone and epidermal growth factor to uterine fibromyomata. Horm Res 32:154–156.
4. Vanni R, Lecca U, Faa G (1991): Uterine leiomyoma cytogenetics II. Report of forty cases. Cancer Genet Cytogenet 53:247–256.
5. Pandis N, Heim S, Bardi G, Floderus UM, Willen H, Mandahl N, Mitelman F (1991): Chromosome analysis of 96 uterine leiomyomas. Cancer Genet Cytogenet 55:11–18.
6. Ozisik YY, Meloni AM, Surti U, Sandberg AA (1993): Deletion of 7q22 in uterine leiomyomas. A cytogenetic review. Cancer Genet Cytogenet 71:1–6.
7. Han K, Lee W, Harris CP, Simsiman RC, Lee K, Kang C, Meisner LF (1994): Comparison of chromosome aberrations in leiomyoma and leiomyosarcoma using FISH on archival tissues. Cancer Genet Cytogenet 74:19–24.
8. Sargent MS, Weremowicz S, Rein MS, Morton CC (1994): Translocations in 7q22 define a critical region in uterine leiomyomata. Cancer Genet Cytogenet 77:65–68.
9. Dal Cin P, Moerman P, Deprest J, Brosens I, Van den Berghe H (1995): A new cytogenetic subgroup in uterine leiomyoma is characterized by a deletion of the long arm of chromosome 3. Genes Chromosom Cancer 13:219–220.
10. Henning Y, Rogalla P, Wanschura S, Frey G, Deichert U, Bartnitzke S, Bullerdiek J (1997): HMGIC expressed in a uterine leiomyoma with a deletion of the long arm of chromosome 7 along with a 12q14–15 rearrangement but not in tumors showing del(7) as the sole cytogenetic abnormality. Cancer Genet Cytogenet 96:129–133.
11. Fletcher JA (1997): Cytogenetic markers in selected gynecological malignancies. In: Human Cytogenetic Cancer Markers. SR Wolman, S Sell, eds. Humana Press, Totowa, pp. 169–202.
12. Vanni R, Marras S, Schoenmakers EFPM, Dal Cin P, Kazmeierczak B, Senger G, Bullerdiek J, van de Ven WJM, Van den Berghe H (1997): Molecular cytogenetic characterization of del(7q) in two uterine leiomyoma-derived cell lines. Genes Chromosom Cancer 18:155–161.
13. Ishwad CS, Ferrell RE, Davare J, Meloni AM, Sandberg AA, Surti U (1995): Molecular and cytogenetic analysis of chromosome 7 in uterine leiomyomas. Genes Chromosom Cancer 14:51–55.
14. Ishwad CS, Ferrell RE, Hanley K, Davare J, Meloni AM, Sandberg AA, Surti U (1997): Two discrete regions of deletion at 7q in uterine leiomyomas. Genes Chromosom Cancer 19:156–160.
15. Sell SM, Altungoz O, Prowse AA, Meloni AM, Surti U, Sandberg AA (1998): Molecular analysis of chromosome 7q21.3 in uterine leiomyoma: analysis using markers with linkage to insulin resistance. Cancer Genet Cytogenet 100:165–168.
16. Mashal RD, Fejzo ML, Friedman AJ, Mitchner N, Nowak RA, Rein MS, Morton CC, Sklar J (1994): Analysis of androgen receptor DNA reveals the independent clonal origins of uterine leiomyomata and secondary nature of cytogenetic aberrations in the development of leiomyomata. Genes Chromosom Cancer 11:1–6.
17. Xing YP, Powell WL, Morton CC (1997): The del(7q) subgroup in uterine leiomyomata: genetic and biologic characteristics. Further evidence for the secondary nature of cytogenetic abnormalities in the pathobiology of uterine leiomyomata. Cancer Genet Cytogenet 98:69–74.
18. Packenham JP, du Manoir S, Schrock E, Risinger JI, Dixon D, Denz DN, Evans JA, Berchuck A, Barrett JC, Devereux TR, Ried T (1997): Analysis of genetic alterations in uterine leiomyomas and leiomyosarcomas by comparative genomic hybridization. Mol Carcinog 19:273–279.
19. Sandberg AA, Bridge J (1994): The Cytogenetics of Bone and Soft Tissue Tumors. R. Landes Company, Austin.
20. Vikhlyaeva EM, Khodzhaeva ZS, Fantschenko ND (1995): Familial predisposition to uterine leiomyomas. Int J Gynaecol Obstet 51:127–131.
21. Neuhausen SL, Marshall CJ (1994): Loss of heterozygosity in familial tumors from three BRCA1-linked kindreds. Cancer Res 54:6069–6072.
22. Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF, Stratton MR (1995): Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12–13. Oncogene 10:1673–1675.
23. Biggs PJ, Wooster R, Ford D, Chapman P, Mangion J, Quirk Y, Easton DF, Burn J, Stratton MR (1995): Familial cylindromatosis (turban tumor syndrome) gene localised to chromosome 16q12–q13: evidence for its role as a tumor suppressor gene. Nature Genet 11:441–443.
24. Biggs PJ, Chapman P, Lakhani SR, Burn J, Stratton MR (1996): The cylindromatosis gene (cyld1) on chromosome 16q may be the only tumor suppressor gene involved in the development of cylindromas. Oncogene 12:1375–1377.
25. Hemminki A, Tomlinson I, Markie D, Jarvinen H, Sistonen P, Bjorkqvist AM, Knuutila S, Salovaara R, Bodmer W, Shibata D, de la Chapelle A, Aaltonen LA (1997): Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nature Genet 15:87–90.
26. Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994):

The 1993–94 Genethon human genetic linkage map. Nature Genet 7:246–339.

27. Chumakov IM, Rigault P, Le Gall I, Bellanne Chantelot C, Billault A, Guillou S, Soularue P, Guasconi G, Poullier E, Gros I, Belova M, Sambucy JL, Susini L, Gervy P, Glibert F, Beaufils S, Bui H, Massart C, Detand MF, Dukasz F, Lecoulant S, Ougen P, Perrot V, Saumler M, Soravito C, Bahouayila R, Cohenakenine A, Barillot E, Bertrand S, Codani JI, Caterina D, Georges I, Lacroix B, Lucotte G, Sahbatou M, Schmit C, Sangouard M, Tubacher E, Dib C, Faure S, Fizames C, Gyapay G, Millasseau P, Nguyen S, Muselet D, Vignal A, Morissette J, Menninger J, Lieman J, Desai T, Banks A, Brayward P, Ward D, Hudson T, Gerety S, Foote S, Stein L, Page DC, Lander ES, Weissenbach J, Lepaslier D, Cohen D (1995): A YAC contig map of the human genome. Nature 377:175–298.
28. Naylor SL, Carritt B, Boileau C, Beroud C, Alexander C, Allderdice P, Alimov A, Ashworth T, Bonifas J, Bugert P, Buys CHCM, Chipperfield MA, Deng H, Drabkin H, Gemmill RM, Grompe M, Joensuu T, Jonasdottir A, Gizatullin R, Krols L, Leach RJ, Lott ST, Killary A, Martinsson T, Messiaen L, O'Connell P, Opalka B, Plaetke R, Sankila EM, Smith DI, Strachen T, van den Berg A, Zabarovsky E (1996): Report of the sixth international workshop on human chromosome 3 mapping 1995. Cytogenet Cell Genet 72:255–270.
29. Marynen P, Kucherlapati R (1996): Report of the third international workshop on human chromosome 12 mapping 1995. Cytogenet Cell Genet 73:1–24.
30. Shimizu N, Antonarakis SE, Van Broeckhoven C, Patterson D, Gardiner K, Nizetic D, Creau N, Delabar JM, Korenberg J, Reeves R, Doering J, Chakravati A, Minoshima S, Ritter O, Cuticchia J (1995): Report of the fifth international workshop on human chromosome 21 mapping 1994. Cytogenet Cell Genet 70:147–182.
31. Scambler PJ (1994): Report of the fourth international workshop on human chromosome 22 mapping 1994. Cytogenet Cell Genet 67:278–294.
32. Zenklusen JC, Thompson JC, Troncoso P, Kagan J, Conti CJ (1994a): Loss of heterozygosity in human primary prostate carcinomas: a possible tumor suppressor gene at 7q31.1. Cancer Res 54:6370–6373.
33. Zenklusen JC, Bieche I, Lidereau R, Conti CJ (1994b): (C-A)n microsatellite repeat D7S522 is the most commonly deleted region in human primary breast cancer. Proc Natl Acad Sci USA 91:12155–12158.
34. Zenklusen JC, Thompson JC, Klein-Szanto AJ, Conti CJ (1995a): Frequent loss of heterozygosity in human primary squamous cell and colon carcinomas at 7q31.1: evidence for a broad range tumor suppressor gene. Cancer Res 55:1347–1350.
35. Zenklusen JC, Weitzel JN, Ball HG, Conti CJ (1995b): Allelic loss at 7q31.1 in human primary ovarian carcinomas suggests the existence of a tumor suppressor gene. Oncogene 11:359–363.
36. Koike M, Takeuchi S, Yokota J, Park S, Hatta Y, Miller CW, Tsuruoka N, Koeffler HP (1997): Frequent loss of heterozygosity in the region of the D7S523 locus in advanced ovarian cancer. Genes Chromosom Cancer 19:1–5.